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Replication of the effect of *SLC2A9* genetic variation on serum uric acid levels in American Indians

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Increased serum uric acid (SUA) or hyperuricemia, a risk factor for gout, renal and cardiovascular diseases, is caused by either increased production or decreased excretion of uric acid or a mix of both. The solute carrier protein 2 family, member 9 (SLC2A9) gene encodes a transporter that mediates urate flux across the renal proximal tubule. Genome-wide association studies have consistently shown the association of single-nucleotide polymorphisms in this gene with SUA in majority populations. American Indian participants of the Strong Heart Family Study, belonging to multigenerational families, have high prevalence of hyperuricemia. We conducted measured genotype analyses, based on variance components decomposition method and accounting for family relationships, to assess whether the association between SUA and SLC2A9 gene polymorphisms generalized to American Indians ($n = 3604$) of this study. Seven polymorphisms were selected for genotyping based on their association with SUA levels in other populations. A strong association was found between SLC2A9 gene polymorphisms and SUA in all centers combined (P-values: 1.3×10^{-31}–5.1×10^{-23}) and also when stratified by recruitment center; P-values: 1.2×10^{-14}–1.0×10^{-5}. These polymorphisms were also associated with the estimated glomerular filtration rate and serum creatinine but not albumin–creatinine ratio. In summary, the association of polymorphisms in the uric acid transporter gene with SUA levels extends to a new population of American Indians.

INTRODUCTION

Uric acid is the end product of purine metabolism in humans and higher primates. Increased serum uric acid (SUA), that is, hyperuricemia is a risk factor for gout and in some cases for nephrolithiasis. As well, it may contribute to cardiometabolic disorders, such as type 2 diabetes,[1] metabolic syndrome,[2,3] cardiovascular disease (CVD) and chronic kidney disease (CKD).[4–8] The prevalence of hyperuricemia and gout seems to have increased in the United States (US) during the past two decades; by 2007–2008, the prevalence of gout was 3.9% and that of hyperuricemia was about 21%.[9] This increased prevalence may be related to concomitant increases in obesity and hypertension along with changes in diet and physical activity. Genetic factors also have a key role in SUA variation. Several twin and family-based studies have found SUA levels to be significantly heritable.[10–12] Genome-wide linkage scans[10–13] and association studies (GWAS)[14–19] in several populations have consistently pointed to the important role of uric acid transporters, particularly solute carrier protein 2 family, member 9 (SLC2A9), in the regulation of SUA. However, the relevance of these genetic variants to minority populations is largely unknown.

Besides genetic factors, environmental factors, particularly diet, disease state and certain medications seem to influence SUA. Epidemiologic and diet studies have shown fluctuations in SUA with increased intake of purine-rich foods, alcohol and fructose intake. Experimental studies have suggested a role for fructose-induced hyperuricemia in the development of insulin resistance, hypertension and renal disease.[6,20] Our preliminary work in the Strong Heart Family Study (SHFS) revealed that SUA exceeded 4 mg/dl in ∼76% of individuals.[12] This is a matter of concern as SUA levels >4 mg/dl have been associated with atherogenesis and stroke in individuals at risk for diabetes or CVD.[21,22] American Indians also have higher rates of albuminuria and of CKD (ie, estimated glomerular filtration rate (eGFR) $< 60 \, \text{ml}/(\text{min}/1.73 \, \text{m}^2)$) than the general US population due primarily to an excess of diabetes and diabetic nephropathy.[23,24] Furthermore, the prevalence of end-stage renal disease (ESRD) and diabetic-ESRD are much higher (two and four times, respectively) in American Indians than in the general US population.[25]

Therefore, we genotyped well-replicated single-nucleotide polymorphisms (SNPs) in *SLC2A9* in the SHFS to assess their generalizability to American Indians. As secondary aims, we also investigated the association of these SNPs with renal phenotypes given the role of SLC2A9 in renal urate transport and the effect of interaction between *SLC2A9* SNPs and environmental factors on SUA levels.

MATERIALS AND METHODS

Study population

The SHFS is a family-based genetic study in American Indians. It is an extension of the Strong Heart Study which is a population-based observational study of CVD and its risk factors in this population. More than 3600 members

of multigenerational families (mean family size 40 individuals, range 5–100) were enrolled from three centers located in Arizona, North and South Dakota and Oklahoma. The Indian Health Service Institutional Review Board and the institutional review boards from the participating centers and institutions approved the SHFS protocol and all subjects gave informed consent. Study design and methods of the SHFS are in accordance with institutional guidelines and have been described previously.[12,26]

Genotyping data for one SNP (see below) used in this study was available through the Population Architecture using Genomics and Epidemiology (PAGE) consortium, which aims to examine putative causal variants across various population groups such as African Americans, Asian Americans and American Indians. The SHFS is a member of the Genetics Epidemiology of Causal Variants Across the Life Course (CALiCo) consortium, which is in turn member of the PAGE consortium.

Phenotyping
During a clinical visit, information related to anthropometry, alcohol intake, medical history and medication use was obtained using a questionnaire. Anthropometric measurements were obtained by standard reference procedures described previously.[12] Blood was collected after an overnight fast and plasma and serum samples were stored at $-80\,^\circ\mathrm{C}$ until analyzed. SUA was assayed in the SHFS central laboratory by the uricase method and serum creatinine by an enzymatic method, both on the Vitros 950 platform (Ortho Clinical Diagnostics, Rochester, NY, USA). Urine albumin content was measured by a sensitive, nephelometric technique.[27] Urine creatinine was measured by the picric acid method.[28] eGFR was computed using the simplified (ie, four-variable) modified diet and renal disease (MDRD) equation, omitting the ethnicity term, as we have reported previously:[29]

$$[\text{eGFR } [\mathrm{ml/(min/1.73\,m^2)}]] = 186 \times \text{serum creatinine } (\mathrm{mg/dl})^{-1.154} \times \text{age (years)}^{-0.203} \times (1.212 \text{ if black}) \times (0.742 \text{ if female})].$$

Dietary assessment
Food intake was measured using a Block 119-item food frequency questionnaire (FFQ).[30,31] The Block FFQ is a widely used, reliable and validated FFQ.[32] The Block database (Block Dietary Systems) was used to calculate the average daily energy and macronutrient intakes of each study participant in this study.[33]

SNP genotyping
Seven SNPs to be genotyped were selected based on their association with SUA levels and replicability across populations. These are rs16890979, rs6832439, rs6449213, rs13131257, rs737267, rs10805346 and rs12498956 (Supplementary Figure 1). Description of all these variants can be found in the NCBI public database (http://www.ncbi.nlm.nih.gov/projects/SNP/). One SNP (rs16890979) was genotyped at the central DNA laboratory of CALiCo Consortium[34] using the TaqMan genotyping assays (Life Technologies, Carlsbad, CA, USA) and the remaining six SNPs were genotyped at the SHFS Genetic Center using the multiplex VeraCode technology from Illumina according to the manufacturer's protocol (Illumina, San Diego, CA, USA). Details of both techniques are reported elsewhere.[35,36] Cluster calls were checked for accuracy and genotypes were exported as text files for further use in association analysis. Replica samples were included as controls for genotyping and allele calling consistency.

Measured genotype analysis
Genotype frequencies for each SNP were estimated allowing for non-independence due to kinship[37,38] and were tested for departures from Hardy–Weinberg equilibrium in the software package, Sequential Oligogenic Linkage Analysis Routines (SOLAR).[37] Population stratification was tested using the quantitative transmission disequilibrium test (QTDT).[39–41] The QTDT is based on the principle that population structure will affect only between-family associations of the genotype with the trait mean. It compares a model in which both between-family and within-family associations are freely estimated to a model in which within-family association component is fixed at 0. The presence of stratification can be assumed when the estimates of between-family is significantly different from within-family components. Estimates of linkage disequilibrium (LD) between SNPs were determined by

calculating pair-wise D' and r^2 statistics. To investigate the association between *SLC2A9* SNPs and SUA levels, we employed a measured genotype analysis (MGA),[42] as implemented in SOLAR.[37] This approach extends the classical variance component-based biometrical model to account for both the random effects of kinship and the main effects of SNP genotypes and has been described in detail in Boerwinkle *et al*.[42] The covariates age, sex, age × sex, body mass index (BMI), eGFR, type 2 diabetes status, self-reported alcohol intake and medications were included in the final model. Study site was used as an additional covariate while analyzing SUA in all centers combined. Details of the medication use are given in Table 1 and Voruganti *et al*.[12]

Bayesian quantitative trait nucleotide analysis
The MGA described above was used to assess association between SUA and each SNP, analyzing one SNP at a time. However, multiple functional variants will often exist within a chromosomal locus, and joint analysis of multiple variants may be more powerful to detect their effects and to establish which of the many polymorphisms within a region are the most likely to be functional. The Bayesian Quantitative Trait Nucleotide (BQTN) approach,[39] which is essentially a Bayesian method, is based on an underlying measured genotype model that permits joint analysis of multiple variants. The BQTN analysis was implemented in SOLAR to statistically identify the most likely functional SNPs associated with a phenotype. This method has been described in detail elsewhere.[37–39]

SNP-by-environment interaction
The association model was extended to include a multiplicative environment-by-SNP interaction term. The interaction between SNPs and non-genetic factors was conducted using a one degree-of-freedom likelihood ratio test of a single interaction term (SNP × E) as implemented in an unconditional logistic regression. SUA was the dependent variable and SNPs were modeled using an additive genetic model (coded as 0, 1 and 2) based on the number of rare alleles. The model included SNP, environmental factor and SNP × environmental factor terms as covariates. Age and sex were added as additional covariates wherever appropriate. Participants were considered as hypertensive if they were on hypertensive medications or had systolic blood pressure $\geq 140\,\mathrm{mm\,Hg}$ or had diastolic blood pressure $\geq 90\,\mathrm{mm\,Hg}$. Similarly, for diabetes status, participants were considered as diabetic (as per ADA crtiteria) if their fasting blood glucose was $\geq 126\,\mathrm{mg/dl}$ or they were taking diabetic medication. Age, protein and sugar intakes were modeled as continuous variables, whereas sex, BMI, alcohol intake, hypertension and diabetes status were modeled as dichotomous variables.

RESULTS
Baseline characteristics of all participants are shown in Table 1. A total of 3604 (60% women) individuals participated in this study. Hyperuricemia, as characterized by SUA > 7 mg/dl in men and > 6 mg/dl in women, was present in 12, 21 and 17% of individuals from Arizona, Dakota and Oklahoma, respectively, with men having higher SUA levels than women in all centers. Of the individual centers, participants from Arizona had higher eGFR, urinary albumin–creatinine ratio (UACR), BMI, waist circumference and percent body fat values even though they were younger than the participants of the other two centers (Table 1). Participants with hyperuricemia were of older age, had lower GFR and higher BMI, waist circumference, blood pressure and triglycerides as well as lower HDL levels.

Association of SLC2A9 SNPs with SUA levels and renal phenotypes
The *SLC2A9* SNPs were strongly associated with SUA levels (Table 2) with *P*-values of 1.0×10^{-5}–1.3×10^{-31}. All centers combined showed the strongest associations followed by Oklahoma, Arizona and Dakotas, with effect sizes of 2.7–6.4%. The overall variance of all SNPs taking the LD structure within the gene into account was 5.4, 4.6, 4 and 5.3% for all centers combined, Arizona, Dakotas and

Table 1 Distribution of SUA levels and cardio-renal-related phenotypes

Phenotype[a]	All	Arizona	Dakotas	Oklahoma
N (% male)	3604 (40)	1215 (38)	1186 (41)	1203 (42)
Serum uric acid (mg/dl)	5.14 (1.5)	4.87 (1.5)	5.34 (1.5)	5.22 (1.5)
Age (years)	39.96 (17.03)	37.21 (16.0)	39.04 (17.1)	43.65 (17.3)
eGFR (ml/(min/1.73 m^2))[b]	99.98 (28.0)	111.42 (31.4)	95.61 (24.6)	92.78 (23.5)
UACR (mg/g)[c]	46.47 (180.5)	61.33 (219.4)	38.84 (151.9)	39.29 (162.1)
BMI (kg/m^2)[d]	32.14 (7.6)	35.13 (8.2)	30.17 (6.8)	31.15 (6.9)
Waist circumference (cm)	104.36 (18.1)	111.54 (18.6)	99.47 (17.0)	101.95 (16.3)
Body fat (%)	37.32 (10.2)	41.43 (9.7)	34.21 (10.0)	36.29 (9.4)
Systolic blood pressure (mm Hg)	122.39 (16.5)	120.80 (16.5)	119.87 (15.3)	126.53 (16.9)
Diastolic blood pressure (mm Hg)	76.19 (11.1)	76.52 (11.3)	75.22 (10.5)	76.84 (11.5)
Total cholesterol (mg/dl)	180.04 (34.8)	173.86 (32.4)	180.95 (35.6)	185.37 (35.5)
High-density lipoprotein cholesterol (mg/dl)	50.59 (14.2)	48.43 (13.7)	50.62 (13.5)	52.77 (14.9)
Triglycerides (mg/dl)	158.96 (93.4)	162.28 (93.6)	150.62 (92.4)	163.80 (93.8)
Hypertensive (%)	31.6	33.9	23.7	37.3
Diabetic (%)	22.7	32.9	14.3	20.5
Medication use (yes %)				
Aspirin	12.9	11.5	15.7	12.1
Sulfonyureas	10	14.7	9.9	5.3
Metformin	7.9	12.1	5.4	6.1
ACE inhibitors	14.5	16.6	11.7	15.3
Beta blockers	4.0	3.5	4.0	4.4
Calcium channel blockers	5.8	6.3	4.5	6.6
Hydrochlorothiazide	7.2	5.9	5.7	10.1

[a]Mean (standard deviation).
[b]Estimated glomerular filtration rate.
[c]Urinary albumin–creatinine ratio.
[d]Body mass index.

Oklahoma, respectively. Their allele frequencies and the effect sizes (proportion of residual phenotypic variance explained by the SNP) are shown in Table 2, and the genotype-specific means of SUA levels are shown in Supplementary Table 1. QTDT for population stratification showed the absence of population stratification for these SNPs. LD pattern showed that there was strong LD (>0.9) (Supplementary Figure 1) between the missense variant rs16890979 and three SNPs rs6832439, rs737267 and rs13131257. In analyses combining all three centers, *SLC2A9* SNPs were also significantly associated with renal function assessed by either serum creatinine or eGFR. However, they were not associated with UACR (Table 3). None of the SNPs were associated with renal phenotypes in individual centers (Supplementary Table 2).

BQTN analysis
The BQTN analysis identifies a SNP or SNPs that are most likely to be functional either by itself or in combination with other SNPs or in high LD with a functional SNP. In the current analyses, SNPs rs10805346 and rs6449213 showed posterior probability of 1, which indicates that these SNPs are most likely to be functional or in high LD with a functional SNP with respect to their effects on SUA.

SNP-by-environment interaction
On the basis of the BQTN results, we found that SNPs rs10805346 and rs6449213 showed high probability of having a functional effect on SUA levels. Therefore, we assessed rs10805346 and rs6449213 for the SNP-by-environment interaction effects on SUA levels (Table 4). Significant interaction effects on SUA levels were found for sex in all centers combined and individual centers. Age and alcohol intake had a significant interaction effect in all centers combined; however,

they were not significant for individual centers. Protein intake showed a significant interaction in Arizona whereas it showed a trend toward significance in all centers combined (Table 4).

DISCUSSION
The major findings of this study are the strong associations of seven *SLC2A9* SNPs, previously described in other populations, with SUA levels in American Indians indicating the generalizability of these associations to a minority population. In addition, we also found a significant association of these *SLC2A9* SNPs with renal function assessed by either serum creatinine or eGFR. A limitation of this study is that we could not test for the association of these variants with gout as we do not have gout prevalence and diagnosis data.

Originally identified as a glucose transporter, SLC2A9 (or GLUT9) was later found to be a key uric acid transporter.[44–46] Studies in rodents and other molecular observations indicate an important role for SLC2A9 in proximal tubular urate absorption.[47,48] Genome-wide (GWAS) and candidate association studies have found strong association of *SLC2A9* SNPs with SUA levels.[14–19] These studies were mainly conducted in Caucasian, African American and Asian populations. However, none of the studies were conducted in American Indians, a population in which the prevalence of hyperuricemia seems to be much higher than other populations.[14–18] The BQTN approach helps statistically narrow down the variants or regions for further molecular and functional analyses by identifying SNP or SNPs that are most likely to be functional or in high LD with a functional SNP. Our BQTN findings demonstrated strong support for rs10805346 and rs6449213 in the variation in SUA levels. These SNPs have been consistently shown to be associated with SUA levels in various GWAS and candidate gene association studies.[14–19] We also

Table 2 Center-specific associations of SUA levels (mg/dl) with SLC2A9 SNP

SNP	Risk allele/ frequency	All Effect size (%)[a]	P-value	Risk allele/ frequency	Arizona Effect size (%)	P-value	Risk allele/ frequency	Dakotas Effect size (%)	P-value	Risk allele/ frequency	Oklahoma Effect size (%)	P-value
rs16890979	A/0.47	4.6	1.3E−31	A/0.55	3.8	3.8E−11	A/0.42	3.2	1.1E−8	A/0.43	5.1	7.8E−13
Post. Prob[b]		0			0.12			0			0	
rs6832439	A/0.47	4.5	7.7E−31	A/0.55	3.8	2.8E−11	A/0.41	3.3	1.5E−8	A/0.47	4.8	7.2E−12
Post. Prob[b]		0			0.09			0			0	
rs6449213	G/0.28	4.5	1.5E−29	G/0.37	3.0	1.6E−11	G/0.20	3.3	2.8E−9	G/0.27	6.4	1.2E−14
Post. Prob[b]		1.0			0.10			1.0			1.0	
rs13131257	A/0.48	4.5	1.8E−29	A/0.56	4.2	5.8E−12	A/0.42	3.3	5.0E−8	A/0.46	4.1	2.6E−10
Post. Prob[b]		0			0.43			0			0	
rs737267	A/0.49	4.4	2.9E−29	A/0.56	4.1	9.1E−12	A/0.45	3.1	4.5E−8	A/0.48	4.4	1.1E−10
Post. Prob[b]		0			0.21			0			0	
rs10805346	A/0.29	4.1	5.4E−28	A/0.21	4.1	2.4E−11	A/0.39	3.3	1.4E−8	A/0.34	2.7	1.6E−8
Post. Prob[b]		1.0			1.0			1.0			0	
rs12498956	C/0.47	3.4	5.1E−23	C/0.43	3.3	5.9E−10	C/0.49	2.1	1.0E−5	C/0.49	4.1	1.1E−9
Post. Prob[b]		0			0.05			0			0	

[a]Effect size – proportion of residual phenotypic variance that is explained by the SNP.
[b]Post. Prob – posterior probability of a functional effect. Posterior probability of >0.95 is considered as statistically significant evidence of functional effect.
Final model included covariates age, sex, age × sex, body mass index (BMI), eGFR, type 2 diabetes status, self-reported alcohol intake and medications.

Table 3 Association of SLC2A9 SNP with renal function in combined centers sample

SNPs	Serum creatinine Effect size[a] (%)	P-value	Estimated GFR Effect size (%)	P-value	Albumin/creatinine ratio Effect size (%)	P-value
rs16890979	0.53	0.0013	0.52	0.0015	0.13	0.066
rs6832439	0.54	0.0017	0.52	0.0019	0.16	0.051
rs6449213	0.27	0.0028	0.22	0.08	0.01	0.639
rs13131257	0.60	0.00007	0.58	0.0009	0.14	0.050
rs737267	0.46	0.004	0.44	0.0044	0.14	0.056
rs10805346	0.68	0.00005	0.69	0.00008	0.14	0.102
rs12498956	0.23	0.058	0.24	0.047	0.07	0.153

[a]Effect size – proportion of residual phenotypic variance that is explained by the SNP.
Final model included covariates age, sex, age × sex, age2 and age2 × sex.

observed that the missense polymorphism rs16890979 and SNPs in high LD with it had zero or very low posterior probability of having a functional effect on SUA levels.

The strong association of *SLC2A9* SNPs with serum creatinine and eGFR but not with UACR appears to be novel and may contribute to understanding the role of uric acid in pathogenesis of CKD. Elevated SUA has been associated with prevalent and progressive loss of renal function, though there has been much controversy regarding its role in pathogenesis given the possibility of confounding due to its association with the metabolic syndrome as well as its renal excretion. We found that alleles of rs16890979, rs6832439, rs737267 and rs13131257, that were associated with lower SUA levels, were associated with higher eGFR. The relationship between uric acid and kidney function seems to be two-sided. On one hand, decline in GFR (kidney function parameter) may lead to elevation of uric acid, on the other hand, increase in uric acid seems to alter glomerular function through renal vasoconstriction and increased *renin* expression.[49] Further, this seems to be dependent on diabetic nephropathy,

despite the high prevalence of type 2 diabetes in our population, since there is no association of the transporter variants with albuminuria. Population studies conducted so far have found a strong association of *SLC2A9* SNPs with SUA levels but not with renal function. Our results are in accord with several recent studies in which SUA was predictive of incident CKD, defined by decrements in eGFR.[50,51] In our study, we found that *SLC2A9* SNPs in individual centers were not associated with renal phenotypes. This may be due to genetic heterogeneity between the study centers which stems from the fact that the three study centers are recruited from local tribal communities that are geographically separate and which do not overlap between centers. Thus, the phenotypic and genotypic heterogeneity, including allele frequency and effect size differences between the three centers, is likely due to both genetic and environmental differences.

SUA is known to vary with age, sex, adiposity, insulin resistance or diabetes and is associated with hypertension as well. In addition to its regulation by renal excretion, it varies with diets such as those rich in purine and fructose which increase SUA, by increasing its precursor pool, while alcohol intake increases SUA by decreasing its excretion. In the Third National Health and Nutrition Examination Survey (NHANES, 1988–1994), intake of meat and seafood was positively associated with SUA whereas dairy intake was inversely associated with SUA.[52] Importantly, consumption of processed and unprocessed red meat was found to be high in our SHFS cohort.[33] Therefore, we tested gene-by-environment interaction for the two polymorphisms that showed the highest probability of functional effect, with eight environmental variables that may affect SUA levels. We found significant interactions with sex, age, alcohol and protein intake. Association with SUA levels was stronger in women than in men replicating previously reported sex differences in individuals of European ancestry.[18,19] By contrast, a study in African Americans and European Americans found only nominal interaction effects for rs16890979 (missense polymorphism) with sex and none with BMI, alcohol intake or diabetes.[15] Sex seems to be the strongest factor in

Table 4 SNP-by-environment interaction effect on SUA levels

Environmental variable	rs10805346				rs6449213			
	Main effects		Interaction effects		Main effects		Interaction effects	
All	β (SE)[a]	P-value	β (SE)[a]	P-value	β (SE)	P-value	β (SE)	P-value
Age	0.28 (0.05)	5.0E−24	0.003 (0.001)	1.9E−2	−0.31 (0.03)	7.4E−28	−0.003 (0.001)	1.9E−2
Sex	0.17 (0.04)	1.3E−6	0.16 (0.04)	2.1E−4	−0.16 (0.04)	9.0E−6	−0.20 (0.04)	8.2E−6
BMI	0.29 (0.03)	7.9E−26	0.028 (0.03)	0.39	−0.31 (0.03)	1.1E−28	−0.013 (0.03)	0.69
Diabetes status	0.27 (0.03)	1.5E−18	0.045 (0.06)	0.45	−0.31 (0.03)	1.4E−22	−0.014 (0.06)	0.81
Hypertension status	0/27 (0.03)	1.3E−16	0.07 (0.05)	0.21	−0.29 (0.03)	2.1E−19	−0.04 (0.05)	0.39
Alcohol intake	0.30 (0.04)	2.9E−14	−0.04 (0.05)	0.44	−0.37 (0.04)	5.7E−20	0.11 (0.04)	0.03
Dietary intake %[b]								
Protein	0.29 (0.03)	8.1E−23	−0.0005 (0.0003)	0.13	−0.30 (0.03)	2.8E−25	0.0006 (0.0004)	0.09
Simple sugars	0.29 (0.03)	5.7E−23	0.0009 (0.002)	0.32	−0.31 (0.03)	1.4E−26	0.002 (0.003)	0.38
Arizona								
Age	0.31 (0.06)	2.6E−8	0.004 (0.003)	0.22	−0.26 (0.04)	6.7E−9	0.00005 (0.003)	0.99
Sex	0.19 (0.08)	1.2E−2	0.23 (0.09)	1.3E−2	−0.12 (0.06)	5.9E−2	−0.17 (0.07)	2.4E−2
BMI	0.30 (0.05)	4.9E−8	0.022 (0.08)	0.78	−0.25 (0.04)	3.7E−8	−0.024 (0.06)	0.70
Diabetes status	0.33 (0.06)	3.0E−7	−0.09 (0.11)	0.40	−0.31 (0.05)	2.8E−9	0.11 (0.09)	0.21
Hypertension status	0.30 (0.07)	3.8E−6	0.016 (0.011)	0.88	−0.24 (0.05)	5.5E−6	−0.03 (0.09)	0.74
Alcohol intake	0.37 (0.08)	4.1E−6	−0.11 (0.10)	0.28	−0.27 (0.07)	9.4E−5	0.027 (0.08)	0.75
Dietary intake %[b]								
Protein	0.29 (0.06)	2.8E−7	−0.001 (0.0006)	0.09	−0.25 (0.05)	4.4E−8	0.0013 (0.0006)	0.02
Simple sugars	0.29 (0.006)	4.5E−7	−0.005 (0.006)	0.37	−0.26 (0.05)	2.3E−8	−0.001 (0.004)	0.82
Dakotas								
Age	0.25 (0.05)	3.7E−8	0.003 (0.002)	0.16	−0.30 (0.06)	2.9E−7	−0.003 (0.003)	0.26
Sex	0.20 (0.06)	5.6E−4	0.039 (0.07)	0.59	−0.13 (0.07)	0.08	−0.30 (0.09)	6.3E−4
BMI	0.25 (0.04)	3.4E−8	0.07 (0.05)	0.19	−0.29 (0.06)	2.2E−7	−0.027 (0.06)	0.68
Diabetes status	0.25 (0.05)	5.1E−7	0.05 (0.11)	0.64	−0.29 (0.06)	2.4E−6	−0.10 (0.15)	0.51
Hypertension status	0.22 (0.05)	1.6E−5	0.14 (0.09)	0.12	−0.31 (0.06)	1.3E−6	0.056 (0.11)	0.63
Alcohol intake	0.24 (0.07)	8.7E−4	0.010 (0.08)	0.91	0.25 (0.04)			
Dietary intake %[b]								
Protein	0.26 (09.05)	5.2E−8	−0.0007 (0.0005)	0.10	−0.28 (0.06)	4.7E−6	0.0002 (0.0008)	0.84
Simple sugars	0.26 (0.05)	3.8E−8	0.0056 (0.005)	0.24	−0.29 (0.06)	1.5E−6	0.0037 (0.005)	0.49
Oklahoma								
Age	0.27 (0.05)	5.0E−8	0.002 (0.002)	0.53	−0.35 (0.05)	1.1E−12	−0.004 (0.002)	0.09
Sex	0.09 (0.06)	0.11	0.23 (0.08)	2.3E−3	−0.21 (0.06)	6.5E−4	−0.17 (0.08)	2.7E−2
BMI	0.27 (0.05)	1.1E−8	−0.09 (0.06)	0.09	−0.35 (0.05)	4.2E−13	0.05 (0.06)	0.34
Diabetes status	0.27 (0.05)	7.7E−7	−0.003 (0.11)	0.98	−0.33 (0.05)	9.7E−10	−0.05 (0.10)	0.63
Hypertension status	0.27 (0.06)	2.8E−06	−0.029 (0.09)	0.75	−0.32 (0.06)	4.1E−8	−0.062 (0.09)	0.48
Alcohol intake	0.24 (0.06)	1.1E−4	0.046 (0.09)	0.61	−0.37 (0.06)	2.6E−9	0.07 (0.09)	0.41
Dietary intake %[b]								
Protein	0.26 (0.05)	1.0E−7	0.0006 (0.0007)	0.39	−0.34 (0.05)	5.6E−12	0.0003 (0.0007)	0.68
Simple sugars	0.28 (0.05)	2.2E−8	−0.007 (0.004)	0.08	−0.34 (0.05)	2.9E−12	0.006 (0.004)	0.12

[a]Beta coefficient (standard error).
[b]Percent of total calories.

our interaction analyses and therefore we analyzed the association of these two SNPs in men and women separately as well as pre- and post-menopausal women. We found it to be strongly associated with SUA in all these groups. The interaction of *SLC2A9* SNPs with sex is a replication of previously reported studies in Caucasians and African Americans.[15,18,19] We found a significant interaction of rs6449213 genotype with alcohol and protein intakes, neither have been previously reported.

In summary, we report strong association of *SLC2A9* SNPs with both SUA levels and eGFR in American Indians. However, given the high rates of hyperuricemia and CKD in American Indians and their contributions to the excess of CVD in this population, further research on the variants affecting these phenotypes may suggest novel therapeutic targets for disease prevention.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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